binding of myosin II induces conformational changes in subdomain 2 and the proteolytic digestion of the D-loop disturbs the motor function of myosin II. However, although as many as 24 classes of myosin have already been found and their *in vivo* roles are completely different, the contribution of the D-loop to actin-myosin interaction has so far been studied only for myosin II.
In this study, to determine whether the D-loop contributes to the interaction with myosin V and if so, in what way it affects its motor function, we prepared actins modified in the D-loop and analyzed the effects of modifications on the motile properties of myosins II and V. We found that the D-loop modifications, namely, the proteolytic digestion with subtilisin and the M47A point mutation, significantly decreased the gliding velocity on myosin II-HMM in an *in vitro* motility assay, due to a weaker generated force. On the other hand, single molecules of myosin V "walked" with the same velocity on both the wild-type and modified actins; however, the run lengths decreased sharply, correlating with a lower affinity of myosin for actin due to the D-loop modifications.These results show that the D-loop strongly modulates the force generation by myosin II and the processivity of myosin V, presumably affecting actin-myosin interaction in the A.M.ADP.P_i state of both myosins. Our findings are important to understand the principles how an actin molecule may regulate diverse *in vivo* functions of various myosin isoforms.

2891-Pos
Spontaneous Oscillations of a Minimal Acto-Myosin System Under Elastic Loading
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Spontaneous mechanical oscillations occur in various types of biological systems where groups of motor molecules are elastically coupled to their environment. By using an optical trap to oppose the gliding motion of a single bead-tailed actin filament over a substrate densely coated with heavy meromyosin molecules, we mimicked this condition in vitro. We show that this minimal acto-myosin system can oscillate spontaneously. Our finding accords quantitatively with a general theoretical framework where oscillatory instabilities emerge generically from the collective dynamics of molecular motors under load.

2892-Pos
Kinetic Characterization of Non-Muscle Myosin IIB Single-Headed Heavy Meromyosin on Single Molecule Level with Optical Tweezers
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Non-muscle myosin IIB (NMIIB) is a cytoplasmic conventional myosin, which plays an important role in development of the brain and heart, and in directed growth cone motility by maintaining cortical tension in motile cells. It forms short bipolar filaments with ~14 myosin molecules on each side of the bare zone. NMIIB is a very slow myosin both in terms of actin-activated ATPase activity and actin translocation capability. Our previous studies showed that the NMIIB is a moderately high duty ratio (at least 20-25%) motor. The ADP release step (~0.35 s-1), of NMIIB is only ~3 times faster than the rate-limiting phosphate release (0.13 ± 0.01 s-1). Because of its slow ADP off-rate, acto-NMIIB has the highest ADP-affinity reported so far for the myosin superfamily (<0.15 μM). To examine the mechanics and kinetics of NMIIB at the single-molecule level we used a dual-beam optical tweezer to perform the "three-bead" assay. The surface-immobilized bead was coated with recombinantly engineered single-headed heavy meromyosin-like (NMIIB-SH-HMM) molecules. We measured the lifetimes of unitary actomyosin interactions and determined the actin-detachment kinetics with varying ATP concentrations. Results showed that at physiological ATP concentration (1 mM), the rate of detachment of acto-NMIIB-SH-HMM interactions was ~0.51 s-1, similar to the ADP release rate and steady-state ATPase rate reported from solution kinetic studies. Decreasing the ATP concentration to 1 μM did not alter this rate of detachment (~0.47 s-1). Also, our results showed that the power-stroke of NMIIB-SH-HMM was ~8 nm. We will discuss our single-molecule results from the perspective of the essential cellular functions of NMIIB in cell locomotion, tension generation and maintenance.

2893-Pos
Myosin-1 Function Extends to Microtubule-Dependent Processes
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Class 1 myosins are motor proteins found on various organelles and at defined structures at the cell periphery, where they play important roles in organelle translocation, intracellular transport, and cytoskeleton organization. Here, we investigated the cellular function of *Dictyostelium* myosin-1C, a long-tailed, monomeric motor with three tail homology domains called TH1, TH2, and TH3. We identified that in addition to its actin-dependent function in endocytotic processes during interphase, in *Dictyostelium* cells the motor co-localizes with centrosomes and spindle microtubules (MT) during mitosis. *In vitro* TIRF microscopy experiments and MT-cosedimentation assays using truncated myosin-1C tail constructs revealed a direct binding of myosin-1C tail to MTs. Only constructs containing TH1 and TH2 bound efficiently to MTs. Moreover, these two domains were sufficient to prevent MT depolymerization at low nanomolar concentrations of myosin-1C tail, while MT formation, i.e. nucleation and elongation, was unaffected. Additionally, we observed myosin-1C tail mediated cross-linking of MTs to F-actin. In cells, myosin-1C constructs lacking the motor domain did not associate with the spindle. This demonstrates that actin-dependent motor function is required for the cell cycle-dependent relocalization of myosin-1C from actin-rich structures at the cell periphery to MT-associated mitotic structures. Cells producing a hydrolysis deficient full-length myosin-1C mutant exhibited reduced growth rates with increased size of nuclei due to defects in spindle alignment and prolonged mitosis. Single kinesin molecule motility assays showed that MT-bound myosin-1C reduced the attachment rate of kinesin-1 to MTs without affecting its velocity and run-length. From this we propose that myosin-1C may regulate MT-dependent motility and MT dynamics. Our cell biological and functional characterization of a long-tailed class 1 myosin shows that myosin function is not limited to the F-actin network but extends to MT associated processes.

2894-Pos
Myo1e Binds Anionic Phospholipids with High Affinity
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Myo1e is a single-headed motor protein that has been shown to play roles in clathrin-mediated endocytosis in HeLa cells (Krendel *et al.* 2007. *FEBS Letters*. 581:644-650) and podocyte function in the kidney (Krendel *et al.* 2009. *J. Am. Soc. Nephrol.* 20:86-94). The myo1e C-terminal tail domain includes a basic region that is required for localization to clathrin-coated vesicles and is homologous to regions of other myosin-1 proteins that have been shown to bind phospholipids. However, the phospholipid binding properties of myo1e have not been examined. We used sedimentation assays and stopped-flow fluorescence to determine the membrane binding affinities and kinetics of a fluorescently labeled recombinant myo1e-tail construct. We found that the myo1e-tail binds tightly ($K_{eff}^{lipid} < 5$ μM) to large unilamellar vesicles (LUVs) containing physiological concentrations of the anionic phospholipids phosphatidylinositol (4,5)-bisphosphate (PIP_2) and phosphatidylserine (PS). Unlike myo1c, myo1e can also bind to physiological concentrations of PS in the absence of PIP_2. While myo1e has a slightly higher affinity for PIP_2 over PS, this selectivity is much less than observed with myo1c, which contains a putative pleckstrin-homology (PH) domain and shows strong specificity for phosphoinositides. Soluble inositol phosphate headgroups, such as inositol (1,4,5)-trisphosphate, can compete with PIP_2 for binding, but the apparent affinity for the soluble inositol phosphate is substantially lower than that for PIP_2. The rate of myo1e attachment to LUVs is similar to that of myo1c, but the rate of detachment from LUVs is slower than that found for myo1c. The high affinity of the myo1e-tail for phospholipids suggests that, *in vivo*, myo1e is strongly attached to membranes where it plays a role in endocytosis and other physiological processes.

2895-Pos
Control of Myosin-I Force Sensing by Alternative Splicing
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Tension sensing by myosin motors is important for numerous cellular processes, including control of force and energy utilization in contracting muscles, transport of cellular cargos, detection of auditory stimuli, and control of cell shape. Myosins have evolved different tension sensitivities tuned for these diverse cellular tasks, thus it is important to determine the mechanisms and regulation of force sensing within the myosin superfamily. In this study, we examined force sensing by the widely expressed myosin-I isoform, myo1b, which is alternatively spliced in its light chain binding domain (LCBD), yielding proteins with lever-arms of different lengths. We found that the step sizes of the myo1b proteins are not linearly related to the number of IQ motifs in the LCBD, suggesting that splicing introduces a structural feature into the LCBD that affects the lever arm size. We also found the actin-detachment kinetics of the splice isoforms to be extraordinarily tension sensitive, with the magnitude of tension sensitivity linearly related to lever arm length. Thus, in addition to regulating step-size, motility rates, and myosin activation, the LCBD is a regulator of force sensing. Finally, we found that myo1b is substantially more